Does Genetic Variation for Copper Resistance Affect Fathead Minnow (*P. promelas*) Toxicity Tests?

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Abstract:

Unexplained variation in the results of aquatic organism toxicity tests is a consistently observed and troubling phenomenon. Possible sources of variation include differences in condition or nutritional status of the population prior to the test, as well as age, density and handling of organisms during the test. One source of variation in test outcomes that is often overlooked is genetic variability within and among stocks of test organisms. The goal of the current study is to assess the relative magnitude of among-stock and within-stock genetic variation for resistance to copper in fathead minnows. Four commercially available stocks of fathead minnows were reared at the US EPA aquatic rearing facility in Cincinnati. Within each stock, mature fish have been paired in spawning chambers with the aim of producing up to 40 full-sib families, divided into approximately 20 paternal half-sib and 20 maternal half-sib progeny groups. Larval fish were exposed to copper at 14-days post hatch, and monitored for mortality in a series of 48-hour time-to-death tests. A Cox proportional hazards survival model was used to describe differences in resistance among and within stocks. The construction of the model included random and fixed effects of direct additive genetic, maternal additive genetic, and full-sib family. Models based on some variables did not explain the data as effectively as others. Common environmental effects were more important sources of variability than maternal genetic effects.

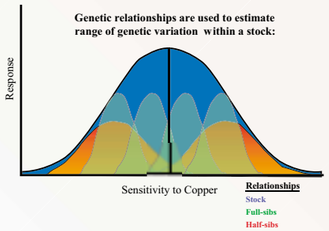
Introduction:

Concerns about the reproducibility of standardized toxicity tests (WET tests, mortality at LC50) continue to be reported (Warren-Hicks et al., 2000,Markle et al., 2000).Variability in test outcomes may be partly due to differences in the diet, culture, test water, and sensitivity of the test species assayed.

We hypothesized that genetic variation within species may also contribute to "within-test error" and reproducibility of aquatic toxicity tests.We used time-to-death tests to assess the genetic variation for resistance to copper in fathead minnows. Time-to-death tests offer more precise information about differences in resistance than what is collected by the use of a single endpoint tests such as LC50.

Sources of Variation are both Environmental and Genetic

Total phenotypic variation is partitioned into genetic and environmental components by analyzing covariation among relatives.



Methods:

I. Four fathead minnow stocks reared in a common environment

- Rearing of parental generation. Fathead minnow stocks were obtained from commercial and governmental suppliers at three months of age and reared for three months at the EPA facility. Food and temperature were optimized to facilitate rapid growth.

Dams and sires rotated in 20 Individual Spawning pens per stock (7 X 12 inches) produced maternal and paternal half-sib families

II. Production of full-sib and half-sib families within each stock

- Spawning cages. Cage mesh was fit to the dimensions of a 12" tall, 7" diameter cylinder, using plastic ties and glue. Fathead minnow densities were reduced prior to pairing to enhance reproductive condition and target potentially active spawners. Spawning tanks were organized with 20-30 spawning baskets in each 55cm X 208cmX 55cm tank. Each cage contained one spawning tile for egg collection.
- Rotation of males among cages. One mating pair was placed into each spawning cage. After approximately 3-5 days (or a spawn event), male fish were rotated into new spawning cages with different females of the same stock.This resulting in female and male fish that spawned multiple times, allowing the creation of a series of maternal and paternal half-sib families. Fish were rotated among cages until a minimum of 20 paternal half-sib groups and 20 maternal half-sib groups were created per stock (approximately 40 full-sib families per stock).
- Rearing of Families. Spawning tiles were checked each morning for egg clutches. Egg cover ranged from 5 to 200 eggs per tile. Tiles with fertile eggs were removed and placed into aerated incubation cups. Fry were reared in the cups for 14 ± 1 days post hatch. Families were reared in separate cups but in a single incubation chamber in order to minimize environmental differences.

	M1	M2	M3
F1	3/24		3/27
F2	4/1	3/27	
F3		3/28	3/25

SAME COLUMN=FULL-SIBS
SAME ROW=HALF-SIBS

Complete set of paternal and maternal half-sib families by 3/28 and 4/1

III. Fathead Minnow time-to-death test

- The protocol for acute toxicity testing followed an approved IACUC Chemical Safety Plan.Water chemistry parameters (DO, temperature) were monitored before and after each test.Analysis of copper concentrations were verified following each exposure experiment.
- A range finding test was performed on 14-day old minnows from one stock. 100% mortality was observed at a copper concentration of 200ug/ L in 96 hours, and 90%mortality was observed at 48 hours.
- Tests were devised so that fish of age 14 ± 1 d were exposed to copper for 48 hours at 200ug/L. For each test, a full-sib family of approximately 20 individuals was apportioned into three exposure cups and one control cup. Dead fish were counted at 20 time points; each hour for the first 9 hours, every 2 hours until 24 hours, then every 4 hours until the end of the test.
- A total of twenty 48-hour tests were performed. Almost all tests included families from each of the four stocks. Most half sibs were exposed in different tests.

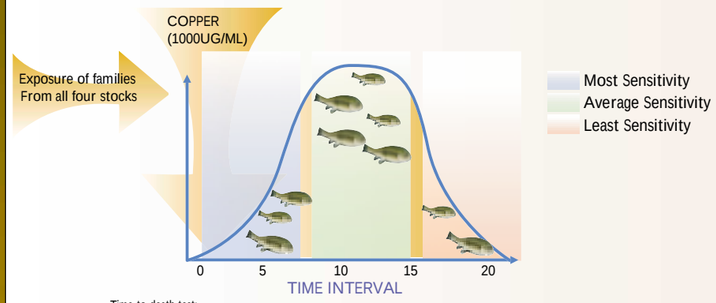
Time to death exposure to 1000ug/L copper for 48 hours

IV. Data analysis

- First step: Evaluate differences in survival among stocks.

Survival Analysis:

Differences in survival were obtained using proportional hazards Survival Kit and PHREG and LIFETEST. The models find percent survival and uses nonparametric, censored data. The test evaluates the risk set of individuals that are dying as a porportion of all that are remaining.



Time-to-death test:
This test measures individual mortality (Dixon and Newman, 1991) and the time for which an individual can tolerate a chemical. Using an LC90 for copper (200 ug/L), individuals were counted at the time of "fatigue" at 20 intervals over a 48 hour test period.

Results:

Cox Proportional Hazard Model

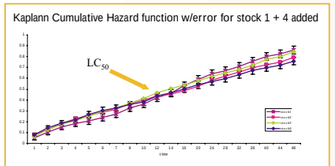
- Percent survival for each stock
- Baseline hazard for each stock

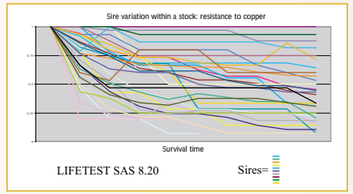
Rationale: assumes no shape, nonparametric, censored data, estimates risk set of individuals as a proportion of all

Table 1. PHREG procedure (SAS 8.20)
Effect of stock and test on time-to-death to copper

Variable	DF	Parameter Estimate	Standard Error	Chi-Square	Pr>ChiSq
Stock	1	-.04292	0.020805	4.2366	0.0396
TEST	1	-.07297	0.00449	263.7056	<.0001

The Cox proportional hazard model is a nonparametric evaluation of the risk of dying in proportion to remaining survivors.





Initial findings:

Stock differences were found to be a significant source of variation in time to death of fathead minnows exposed to copper. However, differences in the average time to death of the best and worst stocks were relatively small. Average failure time of the best performing stock was 13.7 hours, while average failure time of the worst stock was 12.3 hours. In comparison, unexplained test to test variation was a much more important cause of variability in time to death.

Preliminary sire effects within-stocks show large differences in levels of performance.

Next Steps:

We will use the Survival Kit (Ducrocq et al., 2000) program to model random covariates (sire, dam, and stock) and fixed effects with resistance.

Model:

Among-family variance
Covariance of full sibs
$\frac{1}{2}V_A + \frac{1}{4}V_D + V_E c + V_M$

Within-family variance:
$\frac{1}{2}V_A + \frac{3}{4}V_D + V_{EW}$

V_A=additive genetic variance
V_D=dominance variance
$V_E C$= common environmental variance
VM= variance from maternal effect
V_{EW} = error

The estimation of genetic components of resistance to copper within-stocks and between stocks will be further found by estimating proportions dominance, additive genetic, non-additive genetic effects, broad and narrow-sense heritablity.The additional information gained here will quantify if differences in toxicity test outcome are genetic, as well as if test outcomes may be over or under-protective depending on genetic constitution of stocks.

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